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(71) Applicant: F. HOFFMANN-LA ROCHE AG [CH/CH]; 124 Grenzacherstrasse, CH-4070 Basle (CH).

(72) Inventors: ARZENO, Humberto, B.; 7665 Normandy Way, Cupertino, CA 95014-5252 (US). BEARD, Colin, C.; 578 Jefferson Drive, Palo Alto, CA 94303 (US). FISHER, Lawrence, E.; 1036 Marilyn Drive, Mountain View, CA 94040 (US). PRINCE, Anthony; 36 Farm Road, Los Altos, CA 94024 (US).

(74) Agent: MEZGER, Wolfgang; Grenzacherstrasse 124, CH-4070 Basle (CH).

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(54) Title: PROCESS FOR PREPARING PURINE DERIVATIVES

(57) Abstract

Process and novel intermediate for preparing the L-monovaline ester of 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-1,3-propanediol and its pharmaceutically acceptable salts. The present process is a two-step procedure which completely solubilizes the normally insoluble ganciclovir, producing a homogenous solution which can then undergo selective monoesterification. The monoester products are of value as antiviral agents with improved absorption.

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PROCESS FOR PREPARING PURINE DERIVATIVES

BACKGROUND OF THE INVENTION

Field of the Invention

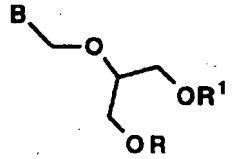
The present invention relates to a process for preparing a prodrug formulation of ganciclovir and its pharmaceutically acceptable salts. More specifically, the invention relates to a process for preparing the L-monovaline ester derived from 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-1,3-propane-diol and its pharmaceutically acceptable salts. The invention also relates to novel intermediates useful in the above process and to a process for preparing those intermediates.

Background Information

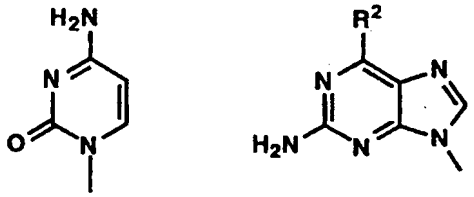
British Patent 1523865 describes antiviral purine derivatives with an acyclic chain in the 9-position. Among those derivatives 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-ethanol or 9-[(1,3-dihydroxy-2-propoxy)-methyl]-guanine (DHPG) with the INN name acyclovir has been found to have good activity against herpes viruses such as herpes simplex.

U.S. Patent 4,355,032 discloses the compound 9-[(2-hydroxy-1-hydroxymethyl-ethoxy)-methyl]-guanine or 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-1,3-propanediol with the INN name ganciclovir. Ganciclovir is highly efficacious against viruses of the herpes family, for example, against herpes simplex and cytomegalovirus. Ganciclovir has the disadvantage of having limited solubility in water.

European Patent Application EP 0 375 329 discloses prodrug compounds with the following formula



wherein R and R^1 are independently selected from a hydrogen atom and an amino acyl residue providing at least one of R and R^1 represents an amino acid acyl residue and B represents a group of the formulae

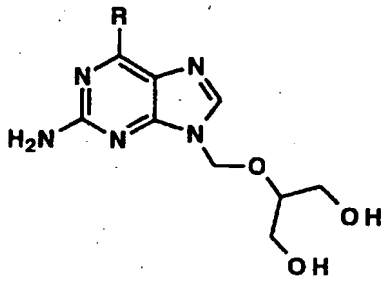


in which R^2 represents a C_{1-6} straight chain, C_{3-6} branched chain or C_{3-6} cyclic alkoxy group, or a hydroxy or amino group or a hydrogen atom and the physiologically acceptable salts thereof. These prodrug compounds are described as having advantageous bioavailability when administered the oral route, resulting in high levels of the parent compound in the body.

Example 3 (b) European Patent Application EP 0 375 329 discloses the preparation of the bis(L-isoleucinate) ester of ganciclovir as a white foam. Example 4 (b) discloses the preparation of the bis(glycinate) ester of ganciclovir as a white solid. Example 5 (b) discloses the preparation of the bis (L-valinate) ester of ganciclovir as a solid. Example 6 (b) discloses the preparation of the bis(L-alaninate) ester of ganciclovir as a syrup containing 90% of the bis ester and 10% of the monoester.

The bis-esters are prepared by reacting ganciclovir with an optionally protected amino acid or functional equivalent thereof; the reaction may be carried out in a conventional manner, for example in a solvent such as pyridine, dimethylformamide, etc., in the presence of a coupling agent such as 1,3-dicyclohexylcarbodiimide, optionally in the presence of a catalytic base such as 4-dimethylaminopyridine. The described bis esters are non-crystalline materials which are difficult to process for the manufacture of oral pharmaceutical dosage forms.

British Patent Application No. 8829571 is the priority patent application for European Patent Application EP 0 375 329 and US Patent No. 5,043,339, and discloses amino acid esters of the compounds of the formula



(wherein R represents a hydroxy or amino group or a hydrogen atom) and the physiologically acceptable salts thereof. Examples of preferred amino acids include aliphatic acids e.g. containing up to 6 carbon atoms such as glycine, alanine, valine and isoleucine. The amino acid esters include both mono and diesters. The preparation of the diesters is identical to the preparation in European Patent Application EP 0 375 329; however, this patent application as well as European Patent Application EP 0 375 329 and US Patent No. 5,043,339 do not disclose the preparation of monoesters, or any data suggesting their

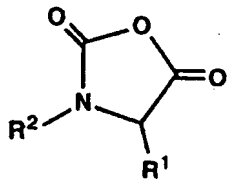
usefulness.

Leon Colla et. al., J. Med. Chem. (1983) 26, 602-604 disclose several water-soluble ester derivatives of acyclovir and their salts as prodrugs of acyclovir. The authors indicate that acyclovir cannot be given as eye drops or intramuscular injections because of its limited solubility in water and have therefore synthesized derivatives of acyclovir which are more water soluble than the parent compound. The authors disclose the hydrochloride salt of the glycyl ester, the hydrochloride salt of the alanyl ester, the hydrochloride salt of the β-alanyl ester, the sodium salt of the succinyl ester, and the azidoacetate ester. The alanyl esters were prepared by conventional esterification methods, including reacting acyclovir with the corresponding N-carboxy-protected amino acid in pyridine, in the presence of 1,3-dicyclohexylcarbodiimide and a catalytic amount of p-toluenesulfonic acid and subsequently catalytic hydrogenation to give the alpha- and beta-alanyl esters as their hydrochloride salts.

L. M. Beauchamp et. al., Antiviral Chemistry & Chemotherapy (1992), 3 (3), 157-164 disclose eighteen amino acid esters of the antiherpetic drug acyclovir and their effectiveness as prodrugs of acyclovir, evaluated in rats by measuring the urinary recovery of acyclovir. Ten prodrugs produced greater amounts of the parent drug in the urine than acyclovir itself: the glycyl, D,L-alanyl, L-alanyl, L-2-aminobutyrate, D,L-valyl, L-valyl, DL-isoleucyl, L-isoleucyl, L-methionyl, and L-prolyl ester. According to the authors the L-valyl ester of acyclovir was the best prodrug of the esters investigated. These esters were prepared by methods similar to those employed by Colla et. al.

European Patent Publication 308 065 discloses the valine and isoleucine esters of acyclovir, preferably in the L-form, as showing a large increase in absorption from the gut after oral administration, when compared with other esters and acyclovir. The amino acid esters are prepared by conventional esterification methods, including reacting acyclovir with an N-carboxy-protected amino acid or an acid halide or acid anhydride of the amino acid, in a solvent such as pyridine or dimethylformamide, optionally in the presence of a catalytic base.

PCT Patent Application WO 94/29311 discloses a process for the preparation of amino acid esters of a nucleoside analogue, including acyclovir and ganciclovir. This process comprises reacting a nucleoside analogue having an esterifiable hydroxy group in its linear or cyclic ether moiety, with a 2-oxa-4-aza-cycloalkane-1,3-dione of the formula



wherein R^1 may represent hydrogen, C_{1-4} alkyl or alkenyl group or other amino acid side chains, and R^2 may represent hydrogen or a group $COOR^3$ where R^3 is a benzyl, t-butyl, fluorenylmethyl or an optionally halo substituted linear or branched C_{1-8} alkyl group. Preferred R^1 groups include hydrogen, methyl, iso-propyl and isobutyl, yielding respectively the glycine, alanine, valine and isoleucine esters of acyclovir or ganciclovir. Examples 1-3 of PCT Patent Application WO 94/29311 discloses only the condensation of acyclovir with the valine-substituted 2-oxa-4-aza-cycloalkane-1,3-dione (Z-valine-N-carboxy-

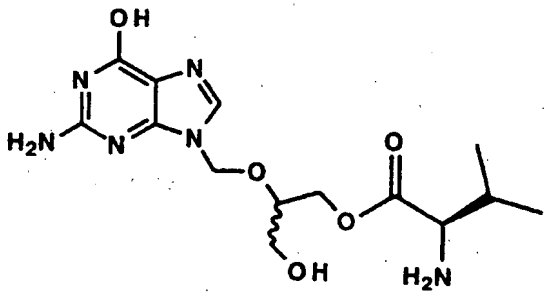
anhydride) by conventional procedures. While the amino acid esters of the PCT application include both the acyclovir and ganciclovir (DHPG) esters, the application does not disclose how to prepare the ganciclovir esters, much less the mono-esters of ganciclovir.

The L-monovaline ester derived from 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)methoxy-1,3-propane-diol and its pharmaceutically acceptable salts are potent antiviral agents and are described in European Patent Application EP 694 547 A. These compounds have been found to have improved oral absorption and low toxicity. This patent application also discloses certain processes for preparing these esters, different from those described herein.

The present invention relates to an improved process for preparing the L-monovaline ester of ganciclovir whereby ganciclovir is reacted with a silane compound to provide a novel ganciclovir intermediate which allows for mono-esterification by an L-valine derivative. This procedure completely solubilizes the normally insoluble ganciclovir, producing an homogenous solution which can then undergo selective monoesterification. What is more, this process is quicker and shorter than the previously known procedures for preparing the L-monovaline ester of ganciclovir.

SUMMARY OF THE INVENTION

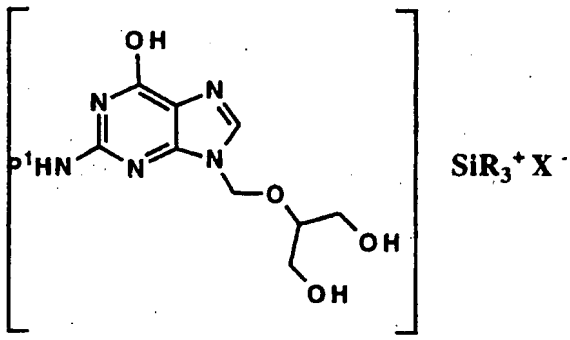
In a first aspect, this invention provides a process for preparing the compound of the formula I:



and pharmaceutically acceptable salts thereof, which compound is named hereinafter 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-3-hydroxy-1-propyl-L-valinate or mono-L-valine ganciclovir.

This process involves the reaction of ganciclovir with a silane compound to provide a soluble ganciclovir intermediate. This intermediate, in turn, allows for mono-esterification by an L-valine derivative, to provide a monovaline ester of ganciclovir, followed by removal of any protecting groups to yield the prodrug of Formula I. Optionally, the process can also include the formation of salts of the prodrug of Formula I, the conversion of an acid addition salt of the prodrug of Formula I into a non-salt form, the optical resolution of a prodrug of Formula I or the preparation of the prodrugs of Formula I in crystalline form. Details of the process are described below.

In a second aspect, this invention provides a compound of Formula III which is a useful intermediate for preparing mono-L-valine ganciclovir and its pharmaceutically acceptable salts:



wherein P^1 is hydrogen or an amino-protecting group and R is alkyl of 1-10 carbon atoms, aralkyl or aryl, and X is a halo, imidazo or an acetamido group.

A third aspect of this invention is a process for preparing the novel intermediate of Formula III.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Unless otherwise stated, the following terms used in the specification and claims have the meanings given below:

"Alkyl" means a straight or branched saturated hydrocarbon radical having from one to the number of carbon atoms designated. For example, C_{1-7} alkyl is alkyl having at least one but no more than seven carbon atoms, e.g. methyl, ethyl, i-propyl, n-propyl, n-butyl, n-pentyl, n-heptyl and the like.

"Lower alkyl" means an alkyl of one to six carbon atoms.

"Aryl" means an organic radical derived from an aromatic hydrocarbon by the removal of one hydrogen atom. Preferred aryl radicals are aromatic carbocyclic radicals having a single ring (e.g., phenyl) or two condensed rings

(e.g., naphthyl).

"Aralkyl" means an alkyl group in which a hydrogen atom is replaced by an above-defined aryl group.

"Acyl" means an organic radical derived from an organic acid by the removal of the hydroxyl group; e.g., CH_3CO- or acetyl is the acyl radical of CH_3COOH. Other examples for such acyl groups are propionyl, or benzoyl, etc. The term "acyl" includes the term "alkanoyl" which is the organic radical RCO- in which R is an alkyl group as defined above.

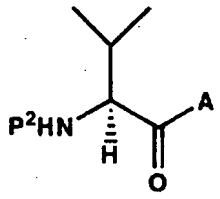
"Lower alkoxy", "(lower alkyl)amino", "di(lower alkyl)amino", "(lower alkanoyl)amino", and similar terms mean alkoxy, alkylamino, dialkylamino, alkanoylamino, etc. in which the or each alkyl radical is a "lower alkyl" as described above.

"Halogen" or "halo" means fluorine, chlorine, bromine, or iodine.

"Derivative" of a compound means a compound obtainable from the original compound by a simple chemical process

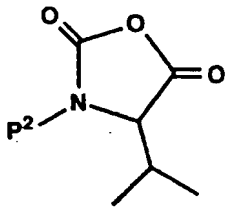
"Activated derivative" of a compound means a reactive form of the original compound which renders the compound active in a desired chemical reaction, in which the original compound is only moderately reactive or non-reactive. Activation is achieved by formation of a derivative or a chemical grouping within the molecule with a higher free energy content than that of the original compound, which renders the activated form more susceptible to react with another reagent. In the context of the present invention activation of the carboxy group is of particular importance and corresponding activating agents or groupings which activate the carboxy group are

described in more detail below. An example of an activated derivative of L-valine is the compound of Formula IV



wherein P^2 is an amino-protecting group and A is a carboxy-activating group, for example, halo , a lower acyloxy group, a carbodiimide group, such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC), an isobutyrate group, and the like.

Of particular interest for the present invention is an amino acid anhydride which is an activated form of an amino acid which renders the amino acid (especially L-valine) susceptible to esterification. Amino acid anhydrides are included in the compounds of Formula IV, above. Especially useful for the present invention are the cyclic amino acid anhydrides of L-valine, described in PCT Patent Application WO 94/29311, such as 2-oxa-4-aza-5-isopropyl-cycloalkane-1,3-dione of formula IVa:



in which P^2 is an amino protecting group. Other examples of the cyclic amino acid anhydrides are protected amino acid N-carboxy anhydrides (NCA's) described in more detail below.

"Protecting group" means a chemical group that (a) preserves a reactive group from participating in an undesirable chemical reaction; and (b) can be easily removed after protection of the reactive group is no longer required. For example, the benzyl group is a protecting group for a primary hydroxyl function.

"Amino-protecting group" means a protecting group that preserves a reactive amino group that otherwise would be modified by certain chemical reactions. The definition includes the silyl group $-SiR_3$,, as defined below, the formyl group or lower alkanoyl groups with 2 to 4 carbon atoms, in particular the acetyl or propionyl group, the trityl or substituted trityl groups, such as the monomethoxytrityl group, dimethoxytrityl groups such as the 4,4'-dimethoxytrityl or 4,4'-dimethoxytriphenylmethyl group, the trichloroacetyl group, the trifluoroacetyl group, and the N-(9-fluorenylmethoxycarbonyl) or "FMOC" group, the allyloxycarbonyl group or other protecting groups derived from halocarbonates such as (C_6-C_{12})aryl lower alkyl carbonates (such as the N-benzyloxycarbonyl group derived from benzylchlorocarbonate), or derived from biphenylalkyl halo carbonates, or tertiary alkyl halo carbonates, such as tertiary butylhalocarbonates, in particular tertiary butylchloro-carbonate, or di(lower)-alkyldicarbonates, in particular di(t-butyl)dicarbonate, the phthalyl group and triphenylmethyl halides such as triphenylmethyl chloride, and trifluoroacetic anhydride.

"Hydroxy-protecting group" means a protecting group that preserves a hydroxy group that otherwise would be modified by certain chemical reactions. In the context of the present invention, the hydroxy-protecting group can be the silane group $-SiR_3$, resulting from the reaction of the silane compound of Formula $XSiR_3$ (Formula II), wherein R is alkyl of 1-10 carbon atoms, aralkyl or aryl, and X is a

halo, imidazo or an acetamido group, with the side chain hydroxy moiety of ganciclovir.

"Leaving group" means a labile group that is replaced in a chemical reaction by another group. Examples of leaving groups are halogen, the optionally substituted benzyloxy group, the isopropyloxy group, the mesyloxy group, the tosyloxy group or the acyloxy group.

All the activating and protecting agents employed in the preparation of the compound of Formula I must meet the following qualifications: (1) their introduction should proceed quantitatively and without racemization of the L-valine component; (2) the protecting group present during the desired reaction should be stable to the reaction conditions to be employed; and (3) the group must be readily removed under conditions in which the ester bond is stable and under which racemization of the L-valine component of the ester does not occur.

The process of the invention may also include the optical resolution of a prodrug of Formula I. Terminology relating to the stereochemistry and optical resolution of these compounds is described in European Patent Application EP 694 547 A.

"Optional" or "optionally" means that a described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, "optionally substituted phenyl" means that the phenyl may or may not be substituted and that the description includes both unsubstituted phenyl and phenyl wherein there is substitution; "optionally followed by converting the free base to the acid addition salt" means that said conversion may or may not be carried out in order for the process described to fall within the

invention, and the invention includes those processes wherein the free base is converted to the acid addition salt and those processes in which it is not.

"Pharmaceutically acceptable" means that which is useful in preparing a pharmaceutical composition that is generally safe and non-toxic and includes that which is acceptable for veterinary use as well as human pharmaceutical use.

"Pharmaceutically acceptable salts" means salts which possess the desired pharmacological activity and which are neither biologically nor otherwise undesirable. Such salts include acid addition salts formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or with organic acids such as acetic acid, propionic acid, hexanoic acid, heptanoic acid, cyclopentane-propionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, o-(4-hydroxy-benzoyl)-benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethane-disulfonic acid, 2-hydroxyethane-sulfonic acid, benzenesulfonic acid, p-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, p-toluenesulfonic acid, camphorsulfonic acid, 4-methyl-bicyclo[2.2.2]oct-2-ene-1-carboxylic acid, gluco-heptonic acid, 4,4'-methylene-bis(3-hydroxy-2-naphthoic)acid, 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxy-naphthoic acids, salicylic acid, stearic acid, muconic acid, and the like. Preferred pharmaceutically acceptable salts are those formed with hydrochloric, sulfuric, phosphoric acid, acetic or methanesulfonic acid, ethanesulfonic acid, 1,2-ethane-

disulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, p-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, p-toluenesulfonic acid, and camphorsulfonic acid.

Synthetic Reaction Parameters

Unless specified to the contrary, the reactions described herein take place at atmospheric pressure within a temperature range from 5°C to 170°C (preferably from 10°C to 50°C; most preferably at "room" or "ambient" temperature, e.g., 20° - 30°C). However, there are clearly some reactions where the temperature range used in the chemical reaction will be above or below these temperature ranges. Further, unless otherwise specified, the reaction times and conditions are intended to be approximate, e.g., taking place at about atmospheric pressure within a temperature range of about 5°C to about 100°C (preferably from about 10°C to about 50°C; most preferably about 20°C) over a period of about 1 to about 100 hours (preferably about 5 to 60 hours). Parameters given in the Examples are intended to be specific, not approximate.

Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification procedure such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography or thick-layer chromatography, or a combination of these procedures. Specific illustrations of suitable separation and isolation procedures can be had by reference to the examples hereinbelow. However, other equivalent separation or isolation procedures can, of course, also be used.

Preferred Embodiments

While the broadest definition of this invention is set forth in the Summary of the Invention as a process for preparing the compound of Formula I and its pharmaceutically acceptable salts, the (R,S) mixture and certain salts are preferred.

The following acids are preferred to form pharmaceutically acceptable salts with the compound of Formula I: hydrochloric, sulfuric, phosphoric acid, acetic, methanesulfonic, ethanesulfonic, 1,2-ethanedisulfonic, 2-hydroxyethanesulfonic, benzenesulfonic, p-chlorobenzenesulfonic, 2-naphthalenesulfonic, p-toluenesulfonic and camphorsulfonic acid. Most preferred are strong inorganic acids, such as hydrochloric, sulfuric or phosphoric acid.

The most preferred compounds are 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-3-hydroxy-1-propyl L-valinate hydrochloride and acetate. These compounds can be prepared as crystalline materials and therefore can be easily manufactured into stable oral formulations.

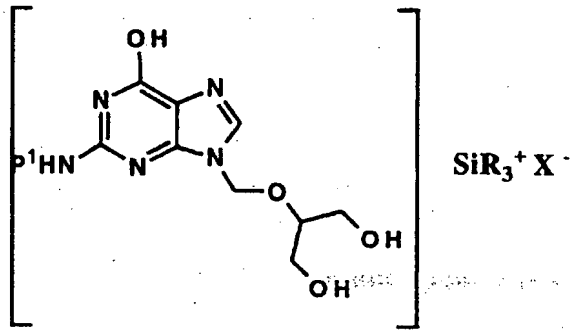
In any of the processes described herein, a reference to Formula I, II, III, IV or IVa refers to such Formulae wherein P^1, P^2, R, X and A are as defined in their broadest definitions set forth in the Summary of the Invention, with the processes applying particularly to the presently preferred embodiments.

Details of the Synthetic Processes

The process of the present invention involves the reaction of 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-1,3-propanediol (ganciclovir) with a silyl compound of Formula II

$XSiR_3$

wherein R is alkyl of 1-10 carbon atoms, aralkyl or aryl, and X is a halo, imidazo or an acetamido group, to yield a soluble ganciclovir intermediate of Formula III



wherein P^1 is hydrogen or an amino-protecting group and R is alkyl of 1-10 carbon atoms, aralkyl or aryl, and X is a halo, imidazo or an acetamido group.

This procedure completely solubilizes the normally insoluble ganciclovir, producing an homogenous solution which can then undergo selective monoesterification with an L-valine derivative of Formula IV or IVa. The resulting monovaline ester of ganciclovir is then subjected to removal of the silyl group, and any protecting groups to afford the compound of Formula I. This process provides the desired mono-L-valine ganciclovir in only two steps.

Compounds of Formula I can optionally be converted into a pharmaceutically acceptable salt thereof. The process can also include the conversion of an acid addition salt of the prodrug of Formula I into a non-salt form, the optical resolution of a compound of Formula I or the preparation of the compound of Formula I in crystalline form.

The process for producing the compound of the Formula I may or may not involve protection of the amino group in the 2-position of the guanine base, though the preferred process is carried out without a protected amino group. For the case when the ganciclovir starting material does have a protected 2-amino group, the protecting group may be removed by conventional procedures, well-known in the art. For example, if the amino-protecting group is a lower alkanoyl group, basic conditions (pH between 8 to 11) are employed to remove the protecting group. For example, 2-N-acetyl ganciclovir is treated with an alkaline reagent such as ammonium hydroxide, sodium or potassium carbonate or sodium or potassium hydroxide until the removal of the acetyl group is complete. In general, this reaction will be conducted in the presence of a suitable solvent such as a lower alkanol. Preferably the starting material is dissolved in methanol and a stoichiometric excess of ammonium hydroxide is added. The reaction temperature is kept between 0 to 50°C, preferably at room temperature. After the reaction is complete (which can be determined by TLC), another solvent may be added to facilitate isolation of the de-protected product, such as ethyl ether which leads to precipitation of the de-acylated product which can be filtered off and isolated using conventional separation methods.

Starting Materials

All starting materials employed to make the compound of Formula I are known, such as ganciclovir, the silane compounds $XSiR_3$ (Formula II), and the protecting and carboxylic-group-activating reagents. Preferred silane compounds are chlorotrimethyl silane and chloro-t-butyldimethylsilane

Prior to carrying out Step II (esterification step), the amino group of the L-valine derivative must be protected to avoid its interference with the esterification by undesirable amide formation. The various amino-protected L-valine derivatives useful in this invention, such as N-benzyloxycarbonyl-L-valine, BOC-L-valine and FMOC-L-valine, N-formyl-L-valine and N-benzyloxycarbonyl-N-carboxy-L-valine anhydride, are all commercially available (SNPE Inc., Princeton, NJ, Aldrich Chemical Co., Milwaukee, WI, and Sigma Chemical Co., St. Louis, MO.), or are described in the literature, such as N-allyloxycarbonyl-L-valine. Cyclic amino-protected L-valine derivatives are also described in the literature, as noted above. Of particular interest for the present invention is the benzyloxycarbonyl valine-substituted 2-oxa-4-aza-cycloalkane-1,3-dione (Z-valine-N-carboxyanhydride, or Z-valine-NCA), which is also commercially available (SNPE Inc., Princeton, NJ). Alternatively, the protecting step may be carried out by conventional methods.

A preferred ganciclovir starting material for the preparation of the compound of the invention is the unprotected ganciclovir (2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-1,3-propanediol) which is described in US Patent No. 4,355,032. Other ganciclovir starting materials may have protection at the 2-amino group, such as 2-(2-acyl-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-1,3-propanediol.

Preparation of Activated derivative of L-valine:

Prior to carrying out Step II (esterification step), L-valine must also be activated. At least 1 equivalent of the protected amino acid and 1 equivalent of a suitable coupling agent or dehydrating agent, for example 1,3-

dicyclohexylcarbodiimide or salts of such diimides with basic groups should be employed from the start. Other carbodiimides such as N,N'-carbonyldiimidazole may also be used. Further useful dehydrating agents are trifluoroacetic anhydride, mixed anhydrides, acid chlorides, 1-benzo-triazolyloxy-tris(dimethylamino)phosphonium hexafluorophosphate, benzotriazole-1-yl-oxy-trispyrrolidinophosphonium hexafluorophophate 1-hydroxybenzotriazole, 1-hydroxy-4-azabenzotriazole, 1-hydroxy-7-azabenzotriazole, N-ethyl-N'-(3-(dimethylamino)-propyl)carbodiimide hydrochloride, 3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine, O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, O-(1H-benzotriazol-1-yl)-1,1,3,3-bis(tetramethylene)uronium hexafluorophosphate or O-(7-azabenzotriazol-1-yl)-1,1,3,3-bis- (tetramethylene)uronium hexafluorophosphate. A description of these coupling agents by L. A. Carpino can be found in *J. Am. Chem. Soc.* 1993, 115, p. 4397-4398.

Also useful for this purpose are urethane-protected amino acid N-carboxy anhydrides (UNCA's) which are an activated form of an amino acid; these have been described by William D. Fuller et.al., *J. Am. Chem. Soc. 1990, 112,* 7414-7416, which is incorporated herein by reference. Other protected amino acid N-carboxy anhydrides are described in PCT Patent Application WO 94/29311 discussed above. In summary, any other reagent that produces an anhydride or another activated derivative of the protected amino acid under mild conditions can be used as the coupling agent.

The amino-protected amino acid is dissolved in an inert solvent such as a halogenated lower alkane, preferably dichloromethane under an inert atmosphere, for example nitrogen, and the coupling agent is added (preferably 1,3-dicyclohexylcarbodiimide). The reaction mixture is stirred at temperatures between 0 and 50°C preferably at about room temperature. The reaction mixture is filtered and the reaction product (the anhydride of the protected amino acid) isolated. The resulting product is dissolved in a dry inert solvent such as dry dimethyl-formamide and placed under nitrogen.

Preparation of Mono-L-valine Ganciclovir

Step I:

Ganciclovir with an optionally protected 2-amino group is reacted with a silane compound $XSiR_3$ (Formula II) to give a soluble ganciclovir intermediate of Formula III.

At least 1 equivalent (preferably 1-2 equivalents, most preferably 1.2-1.5 equivalents) of the silyl reagent is added to a suspension of ganciclovir in an inert solvent, preferably an aprotic polar solvent, at a temperature of 0-10°C followed by the addition of at least 1 equivalent (usually 1.2-1.5 equivalents) of an organic base such as imidazole, trimethylamine (TMA), piperidine or pyridine, most preferably imidazole.

Step II:

In this step an activated derivative of amino-protected L-valine of the Formula IV or IVa is esterified with the ganciclovir intermediate obtained in Step I. Suitable amino-protecting groups for the L-valine derivative are the N-benzyloxycarbonyl group, the phthalyl group, the tertiary butyloxycarbonyl group and the N-(9-fluorenylmethoxycarbonyl) or "FMOC" group.

A solution of the monosilyl-protected product of Step I is added to an approximately equivalent amount of the activated L-valine derivative, preferably Z-valine-N-carboxyanhydride or L-valine anhydride. The reaction mixture is stirred at 10°-40°C, preferably at ambient temperature for 10-90 hours, preferably about 72 hours.

Step III:

The silyl group can be removed easily after completion of the previous steps by acidic hydrolysis in a manner well-known to those skilled in the art. The hydrolysis reaction is preferably carried out by treating the silyl mono-valine ganciclovir intermediate obtained in Step II with dilute aqueous acid, preferably dilute aqueous hydrochloric acid.

Step IV:

The valine amino-protecting group in the product of Step III is removed by a de-protection reaction, preferably in an acidic medium or solvent, most preferably by hydrogenolysis. De-protection under acidic conditions is preferred, as this will ensure that the amino group liberated in the de-protection reaction will be protonated; that is, that the base of Formula I as it is formed in the de-protection reaction will be captured by an at least stoichiometric amount of acid present. Isolating the compound of Formula I as an acid addition salt will protect the desired stereoconfiguration of the compound of Formula I. Therefore, those examples given below that show the de-protection step also show the concomitant salt formation step.

The de-protection reaction is carried by dissolving the product of the previous step in an inert solvent, preferably in an acidic solvent, using a hydrogenation

catalyst, such as palladium hydroxide on carbon, palladium on carbon, or platinum, using elevated hydrogen pressure between 1 and 2000 psi (0.07-140 atm), preferably 50 to 200 psi (3.5-14 atm), most preferably 5 to 20 psi (0.35-1.4 atm). The completion of the reaction can be monitored using conventional TLC analysis. The hydrogenolysis is continued until the conversion is complete, if required with addition of further hydrogenation catalyst. The catalyst is removed and washed. The combined filtrates from filtration and the washings are concentrated and lyophilized to isolate ganciclovir L-valine ester. The purification of the product and the isolation of a crystalline ester is carried out by recrystallization or other purification techniques, such as liquid chromatographic techniques.

If the tertiary butyloxycarbonyl group is being used as amino-protecting group, its removal is effected with acid, such as HCl and isopropanol as a solvent or with trifluoroacetic acid neat.

Preparation of Salts

One of ordinary skill in the art will also recognize that the compound of Formula I may be prepared as an acid addition salt or as the corresponding free base. If prepared as an acid addition salt, the compound can be converted to the free base by treatment with a suitable base such as ammonium hydroxide solution, sodium hydroxide, potassium hydroxide or the like. However, it is important to point out that the free base of Formula I is more difficult to characterize than its acid addition salts. When converting the free base to an acid addition salt, the compound is reacted with a suitable organic or inorganic acid (described earlier). These reactions are effected by treatment with an at least stoichiometric

amount of an appropriate acid (in case of the preparation of an acid addition salt) or base (in case of liberation of the free compound of Formula I). In the salt-forming step of this invention, typically the free base is dissolved in a polar solvent such as water or a lower alkanol (preferably isopropanol) and mixtures thereof and the acid is added in the required amount in water or in lower alkanol. The reaction temperature is usually kept at about 0 to 50 °C, preferably at about room temperature. The corresponding salt precipitates spontaneously or can be brought out of the solution by the addition of a less polar solvent, removal of the solvent by evaporation or in a vacuum, or by cooling the solution.

Isolation of Stereoisomers and the Manufacture of Crystalline 2-(2-Amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-3-hydroxy-1-propyl-L-valinate

From the Formula (I) it is apparent that the compound of the invention has one asymmetric carbon atom (chiral center) in the propanyl chain, in addition to the asymmetric carbon atom in L-valine. Therefore, two diastereomeric forms exist, the (R)- and (S)- form as determined by the rules of Cahn et al. Suitable methods for the separation of the diastereomers are described in European Patent Application EP 694 547 A.

The compounds of Formula (I) may also be prepared in crystalline form, which has many well-known advantages over the non-crystalline form. Suitable methods for the preparation of the compounds of the invention in crystalline form are also described in European Patent Application EP 694 547 A.

The following preparations and examples are given to enable those skilled in the art to more clearly understand

and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

EXAMPLE 1

Preparation of 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-3-hydroxy-1-propyl-N-(benzyloxycarbonyl)-L-valinate

To a suspension of 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-1,3-propanediol (ganciclovir, 10 kg, 39.2 moles) in dimethylformamide (25 l) at -5° to 0°C under an atmosphere of nitrogen was added chlorotrimethylsilane (5.1 kg, 47.0 moles) at such a rate that the temperature of the slurry did not exceed 10°C. Once the addition was finished, the slurry was stirred at 0°C for 3 hours. To this slurry/solution was added imidazole (3.2 kg, 47.0 moles) in one portion. After stirring an additional 30 minutes, the reaction mixture was added to Z-valine-NCA (10 kg, 36.0 moles) over a period of 15 minutes. This mixture was allowed to stir for a period of 72 hours under a nitrogen atmosphere at 21°-23°C.

The reaction mixture was then slowly added to a well stirred solution of 3M HCl (133 l) and CH_2Cl_2 (120 l) at such a rate that the internal reaction temperature did not exceed 25°C. The stirring was stopped and the layers were allowed to separate. The organic layer was collected in a polyethylene drum. The remaining aqueous fraction was extracted successively with 38, 19, 15 and 15 l of CH_2Cl_2. All of the organic fractions were collected in the clean polyethylene drum and reserved. The aqueous fraction was then made basic to pH 1.5 with NH_4OH. The precipitate resulting from this step was collected by filtration. The filter cake so formed was washed twice with H_2O (2X20 l). This solid was dissolved in 3M HCl (133 l)/ CH_3OH (90 l)

and this solution was added to a well stirred solution of the collected organic fractions from the first extraction. Stirring was stopped after 5 minutes and the layers were allowed to separate. The aqueous layer was collected and cooled to 5°C. The organic phase was re-extracted three more times with 3M HCl/CH_3OH (66 l/ 45 l for the first extraction, 33 l/ 22 l for the second extraction, 30 l/ 25 l for the third extraction). The combined aqueous fractions were cooled to 5°C and treated with NH_4OH until a pH of 5.5 was attained (21.4 kg of NH_4OH). The resulting precipitate was collected by filtration. The filtercake so collected was washed with H_2O (2X20 l) and dried under a stream of nitrogen. The solid was placed in a vacuum oven at 55°C and a vacuum of 25 ins was maintained with a nitrogen bleed. After 14 hours in the oven, the product, 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-3-hydroxy-1-propyl-N-(benzyloxycarbonyl)-L-valinate, (or N-Z-ganciclovir monovalinate) had reached a constant weight: 6.7 kg. Purity: (HPLC) 97.8%; H_2O content: 2.1% water by the Karl Fisher test.

EXAMPLE 2

Preparation of 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-3-hydroxy-1-propyl-L-valinate hydrochloride

A vigorously stirred suspension of $Pd(OH)_2/C$ (670 g) in CH_3OH (23 l) was treated with H_2 gas (7 psi [0.49 atm]) for 12 hours. To this suspension was added a solution of N-Z-ganciclovir-monovalinate, 6.7 kg, 13.7 moles) in CH_3OH (34 l) containing concentrated HCl (1.64 kg). The H_2 atmosphere was maintained at 7 psi [0.49 atm]. The atmosphere was replaced at 20 minute intervals. After 2.75 hours, the H_2 atmosphere was replaced with nitrogen. The catalyst was removed by filtration through Solka Floc. The filtrate was concentrated *in vacuo* to approximately 13

1, at which time H_2O (4 l) was added. The volume of the filtrate was again reduced to approximately 13 l. The temperature of the mixture was adjusted to approximately 38°C and isopropyl alcohol (24 l) was slowly added. After crystallization had occurred, the mixture was cooled to 21°C over a period of 2 hours. Additional isopropyl alcohol (24 l) was added and the mixture was stirred for 16 hours at 5°C. The solid was then collected by filtration. The filtercake was washed with cold isopropyl alcohol (19 l) and dried under a stream of nitrogen for 3 days. The solid product, 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-3-hydroxy-1-propyl-L-valinate hydrochloride, was placed in a vacuum oven (50°-55°C, nitrogen bleed to maintain 25 in vacuum). Isopropyl alcohol was found to be 0.4% after 24 hours. Weight of solid: 4.35 kg. Purity: (HPLC) 98.6%; MS: 355 $(MH)^+$.

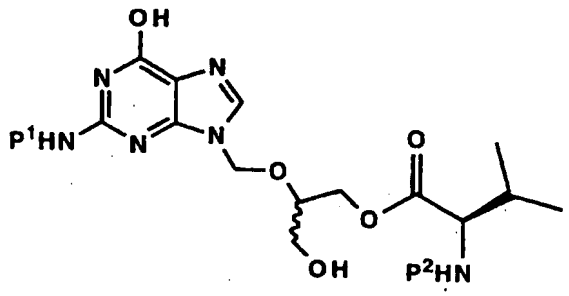
Claims

1. A process for preparing the compound 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-3-hydroxy-1-propyl-L-valinate or a pharmaceutically acceptable salt or diastereomers thereof which process comprises:

(a) esterifying a soluble mono silyl derivative of an optionally 2-N-protected 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)methoxy-1,3-propanediol (ganciclovir) or a salt thereof, with an activated derivative of L-valine in N-protected form;

(b) removing the silyl group from the compound prepared in step (a);

(c) removing any amino-protecting group from a compound with the formula



wherein P^2 is an amino-protecting group, and P^1 is hydrogen or P^2 to afford the compound 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-3-hydroxy-1-propyl-L-valinate or a pharmaceutically acceptable salt thereof;

(d) optionally converting the compound 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-3-hydroxy-1-propyl-L-valinate into a pharmaceutically acceptable salt thereof; or

(e) optionally converting the acid addition salt of the compound 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-3-hydroxy-1-propyl-L-valinate to a non-salt form; or

(f) optionally diastereoisomerically separating 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-3-hydroxy-1-propyl-L-valinate into its (R) and (S) diastereoisomers.

2. The process of Claim 1 wherein the soluble silyl derivative is prepared by reacting 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-1,3-propanediol (ganciclovir) or a salt thereof, with a silane compound of Formula $XSiR_3$, wherein R is alkyl of 1-10 carbon atoms, aralkyl or aryl, and X is a halo, imidazo or an acetamido group.

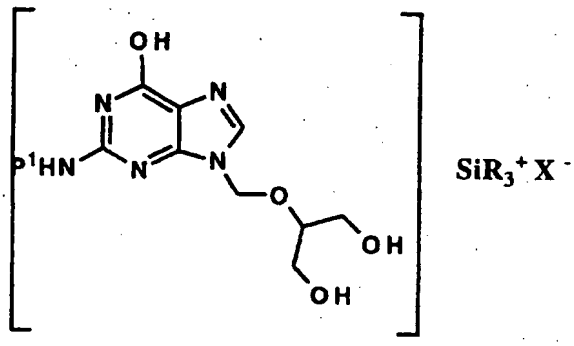
3. The process of Claim 2 wherein the reaction is carried out in the presence of an organic base, preferably wherein the base is pyridine, trimethylamine or imidazole, most preferably imidazole.

4. The process of Claim 2 wherein the silane compound is chlorotrimethyl silane or chloro-t-butyl-dimethylsilane.

5. The process of Claim 2 wherein the reaction is carried out in the presence of at least 1 equivalent of the silane compound, preferably 1-2 equivalents, most preferably 1.2-1.5 equivalents.

6. The process of Claims 1 to 5 wherein the activated derivative of L-valine is Z-valine-N-carboxy-anhydride.

7. A compound of the formula



wherein P^1 is hydrogen or an amino-protecting group and R is alkyl of 1-10 carbon atoms, aralkyl or aryl, and X is a halo, imidazo or an acetamido group.

8. A process for preparing the compound of Claim 7 which process comprises reacting 2-(2-amino-1,6-dihydro-6-oxo-purin-9-yl)-methoxy-1,3-propanediol (ganciclovir) or a salt thereof, with a silane compound of Formula $XSiR_3$, wherein R is alkyl of 1-10 carbon atoms, aralkyl or aryl, and X is a halo, imidazo or an acetamido group.

9. The process of Claim 8 wherein the reaction is carried out in the presence of an organic base, preferably wherein the base is pyridine, trimethylamine or imidazole, most preferably imidazole.

10. The process of Claim 8 wherein the silane compound is chlorotrimethyl silane or chloro-t-butyldimethylsilane.

11. The process of Claim 8 wherein the reaction is carried out in the presence of at least 1 equivalent of the silane compound, preferably 1-2 equivalents, most preferably 1.2-1.5 equivalents.

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INTERNATIONAL SEARCH REPORT

Inter ..al Application No
PCT/EP 97/00228

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D473/00 C07F7/12 C07F7/10 C07F7/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WO 94 29311 A (FARMHISPANIA SA) 22 December 1994 cited in the application see the whole document	1-3
A	US 5 043 339 A (LILIA M. BEAUCHAMP) 27 August 1991 cited in the application	1-3
A	EP 0 375 329 A (THE WELLCOME FOUNDATION LIMITED) 27 June 1990 cited in the application see the whole document	1-3

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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2

Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Luyten, H

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Information on patent family members

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